Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich

Musterlösung Übung 10

Vorlesung Bio-Engineering

Frühlingssemester 2019

Foundations of Cell-Matrix Mechanobiology

I. TRUE/FALSE Decide whether each of these statements is true or false, and then explain why.

Q1: The extracellular matrix is a relatively inert scaffolding that stabilizes the structure of tissues.

A1: False. The extracellular matrix plays an active role influencing the development, migration, proliferation, shape, and metabolism of cells that contact it.

Q2: One of the main chemical differences between proteoglycans and other glycoproteins lies in the structure of their carbohydrate side chains: proteoglycans mostly contain long, unbranched polysaccharide side chains, whereas other glycoproteins contain much shorter, highly branched oligosaccharides.

A2. True. In addition to these differences, proteoglycans can contain as much as 95% carbohydrate by weight, whereas other glycoproteins generally contain a lower fraction of carbohydrate (1–60%).

II. CALCULATIONS

Q3: Platelets are flat, disc-like cells about 2 μm in diameter. Estimates of the number of integrin molecules on their surface vary around a mean of about 80,000. If the integrins themselves are about 10 nm in diameter, how tightly packed are they? (Assume that the total membrane area is $2\pi r^2$.)

A3: Assuming that the area of a platelet can be approximated as the areas of two circles, each 2 mm in diameter, the surface area of a platelet is $2\pi\ 1^2$ = 6.3 μm^2, which is 6.3 x 10^6 nm^2. At 80,000 integrins per platelet, each integrin occupies 78.8 nm^2 (6.3 x 10^6 nm^2 /80,000 integrins). Assuming that each integrin is 10 nm in diameter, the cross-sectional area of an integrin is 78.5 nm^2 (π x5^2). With the assumptions stated in this problem, then, integrins would be very crowded in the membranes of platelets. Regardless of the specific assumptions, integrins clearly occupy a large fraction of the surface area of platelets, as befits their critical role in platelet function.

III. DEFINITIONS Define or briefly explain the following terms:

Q4: Terms to be defined	**A4: Definitions**
anchorage dependent cell behaviors	Dependence of cell survival, growth, and/or proliferation on attachment to a substratum.
An integrin and its ligand	The principal receptor on animal cells for binding most extracellular matrix proteins, including collagens, fibronectin, and laminins.
focal adhesion kinase (FAK)	Focal adhesions are protein complexes that form between and connect the cytoplasmic tails of integrins and the actin cytoskeleton. Cytoplasmic kinases are present at these cell–matrix

	junctions, and are physically bound and released as part of cell-matrix signaling.

IV. MIX AND MATCH Select the best matched term from the list to the provided description in the table.

TERMS TO LEARN: collagen; glycosaminoglycan (GAG); collagen fibril; hyaluronan; elastin; matrix metalloprotease; proteoglycan; fibril-associated collagen; RGD sequence; fibrillar collagen; fibronectin; extracellular matrix; chondroblast; fibroblast; osteoblast; osteoclast; osteocyte;

A5: Best suited term from the list:	**Q5: Description:**
Collagen	Fibrous protein rich in glycine and proline that, in its many forms, is a major component of the extracellular matrix and connective tissues.
Extracellular matrix	Complex network of polysaccharides (such as glycosaminoglycans or cellulose) and proteins (such as collagens) secreted by cells that serves as a structural element in tissues and also influences tissue development and physiology.
Glycosaminoglycan (GAG);	General name for long, linear, highly charged polysaccharides composed of a repeating pair of sugars, one of which is always an amino sugar, that is found covalently linked to a protein core in the extracellular matrix.
Fibrillar collagen	Type of collagen molecule that assembles into ropelike structures and larger, cablelike bundles.
Fibronectin	Extracellular matrix protein that binds to cell-surface integrins to promote adhesion of cells to the matrix and to provide guidance to migrating cells during embryogenesis.
Elastin	Hydrophobic protein that forms extracellular extensible fibers that give tissues their stretchability and resilience.
Fibroblast	Common cell type in connective tissue that secretes an extracellular matrix rich in collagen and other extracellular matrix macromolecules.

V. Experimental Design and Analysis:

Binding of fragments and competition for binding can be used to identify the portion of a larger ligand that is critical for binding. Fibronectin, which is a large glycoprotein component of the extracellular matrix, binds to fibronectin receptors on cell surfaces. Fibronectin can stick cells to the surface of a plastic dish, to which they would otherwise not bind, forming the basis of a simple binding assay. By attaching small fragments of fibronectin to dishes, researchers identified the cell-binding domain as a 108-amino acid segment about three-quarters of the way from the N-terminus.

Synthetic peptides corresponding to different portions of the 108-amino acid segment were tested in the cell-binding assay to precisely localize the active binding region. Two experiments were conducted.

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Experiment 1: first, peptides were linked covalently to plastic dishes via a disulfide bond to an attached carrier protein, and then tested for their ability to promote cell sticking (Table E1).

Experiment 2: Plastic dishes were coated with native fibronectin, and cells that stuck to the dishes in the presence of the synthetic peptides were counted (Table E2).

Table E1. Fibronectin-related peptides tested for their ability to promote cell sticking

PEPTIDE	SEQUENCE	CONCENTRATION REQUIRED FOR 50% CELL ATTACHMENT (nM)
Fibronectin		0.10
Peptide 1	YAVTGRGDSPASSKPISINYRTEIDKPSQM(C)*	0.25
Peptide 2	VTGRGDSPASSKPI(C)	1.6
Peptide 3	SINYRTEIDKPSQM(C)	>100
Peptide 4	VTGRGDSPA(C)	2.5
Peptide 5	SPASSKPIS(C)	>100
Peptide 6	VTGRGD(C)	10
Peptide 7	GRGDS(C)	3.0
Peptide 8	RGDSPA(C)	6.0
Peptide 9	RVDSPA(C)	>100

*The (C) at the C-terminus indicates the cysteine linkage to the carrier protein.

Table E2. Fibronectin-related peptides tested for their ability to block cell sticking.

PEPTIDE	PERCENT OF INPUT CELLS STICKING
GRGDSPC	2.0
GRGDAPC	1.9
GKGDSPC	48
GRADSPC	49
GRGESPC	44
None	47

Q6. The two experiments used different assays to detect the cell-binding segment of fibronectin. Does the sticking of cells to the dishes mean the same thing in both assays? Explain the difference between the assays.

A6. The sticking of cells to the dishes means opposite things in the two experiments. In the first experiment, cell sticking indicates that the peptide is active. In the second experiment, it means the peptide is inactive. In the first experiment, the peptides are stuck to the dish. Only when the peptides contain the active segment will the cells stick to the peptides and, hence, to the dish. In the second experiment, the cells will stick to the fibronectin on the dish unless the receptor sites on the cell surface are already occupied by the small peptide, in which case binding to the dish will be inhibited. The two experiments represent alternative ways of measuring the same thing, namely, the specific interaction between a receptor on the cell surface and a ligand in the cell's environment.

Q7. From the results in Tables E1 and E2, deduce the amino acid sequence in fibronectin that is recognized by the fibronectin receptor.

A7. In the first experiment, the only segments that show activity are those that contain the **tripeptide RGD** (arginine-glycine-aspartic acid). Test "peptide 9" shows that changes in this short sequence abolish the binding activity: substituting the bulky valine (V) for the compact glycine (G) inactivates the peptide. The results of the second experiment confirm those of the first and suggest that the **RGD** sequence is stringently required for activity. Even the conservative substitution of lysine (K) for arginine (R), or of glutamic acid (E) for aspartic acid (D), abolishes the binding.

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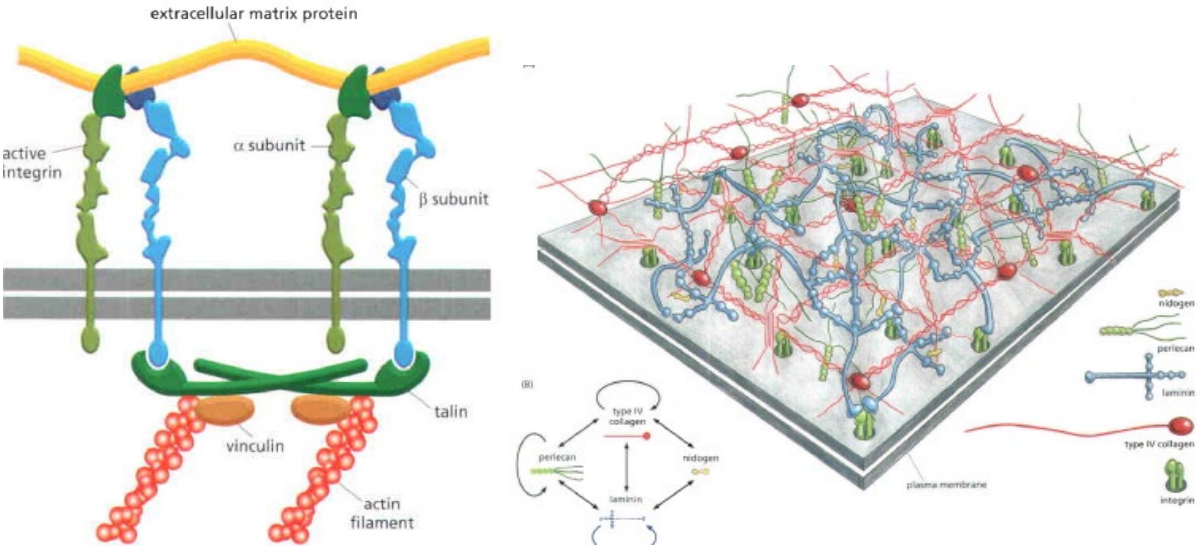
Q8. Cell matrix linking via integrin - TRUE/FALSE (Decide whether each of these statements is true or false, and then explain why.)

a) True. RGD is a small amino acid responsible for integrin binding of fibronectin.

b) Wrong. Inside the cell integrin binds to ~~vinculin~~ talin, which binds to actin filament.

c) True. Inside out and outside in activation have the same result of strong binding.

d) Wrong. ECM protein network consists of nidogen, type ~~III~~ IV collagen, laminin and perlecane.



Q9. Nuclear deformation and related signaling – TRUE/FALSE (Decide whether each of these statements is true or false, and then explain why.)

a) Wrong. Integrin senses mechanical stimuli from ~~neighboring cells~~ the ECM.

b) Wrong. Chromatin ~~is the main cytoskeletal filament in the nucleus.~~ Chromatin is the genetic material which forms the Chromosomes. Chromatin is a complex from DNA and specific proteins.

c) True. Nuclear deformation and molecular mechanisms are both mechanisms of nuclear mechanosensing.